

Laboratory Issues and West Nile Virus

Hema Kapoor MD. SM (NRM)

Virology Section manager

Bureau of laboratories

Michigan Department of community Health

Lansing Michigan

Laboratory Issues and West Nile Virus

- Arboviruses
- Laboratory Diagnosis
- Investigation of 2002 WNV outbreak in Michigan
- Plans for 2003-Lab perspective

The arboviruses

- **Ar**thropod-**b**orne viruses (mosquitoes, sand-flies, fleas, ticks, lice, etc)
- Enveloped RNA viruses -4 families
- **Flaviviridae-WNV** isolated in 1937 in west Nile district of Uganda in Eastern Africa.

Arboviral encephalitides

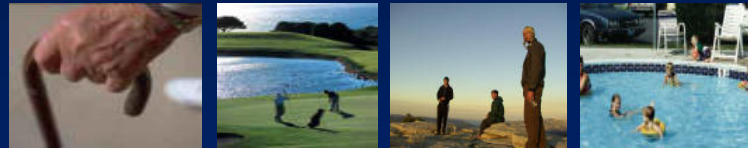
Mosquito

- eastern equine encephalitis (EEE)
- western equine encephalitis (WEE)
- St. Louis encephalitis (SLE)
- La Crosse (LAC)

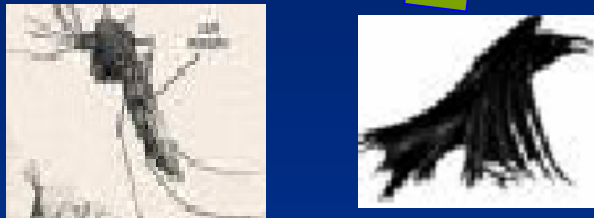
Ticks

- Powassan

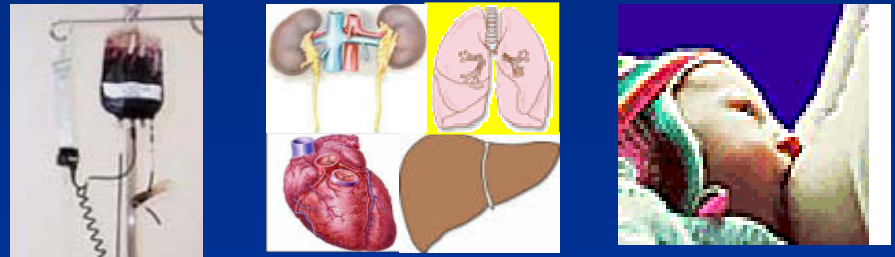
WNV Transmission



Dead end host



New modes of transmission

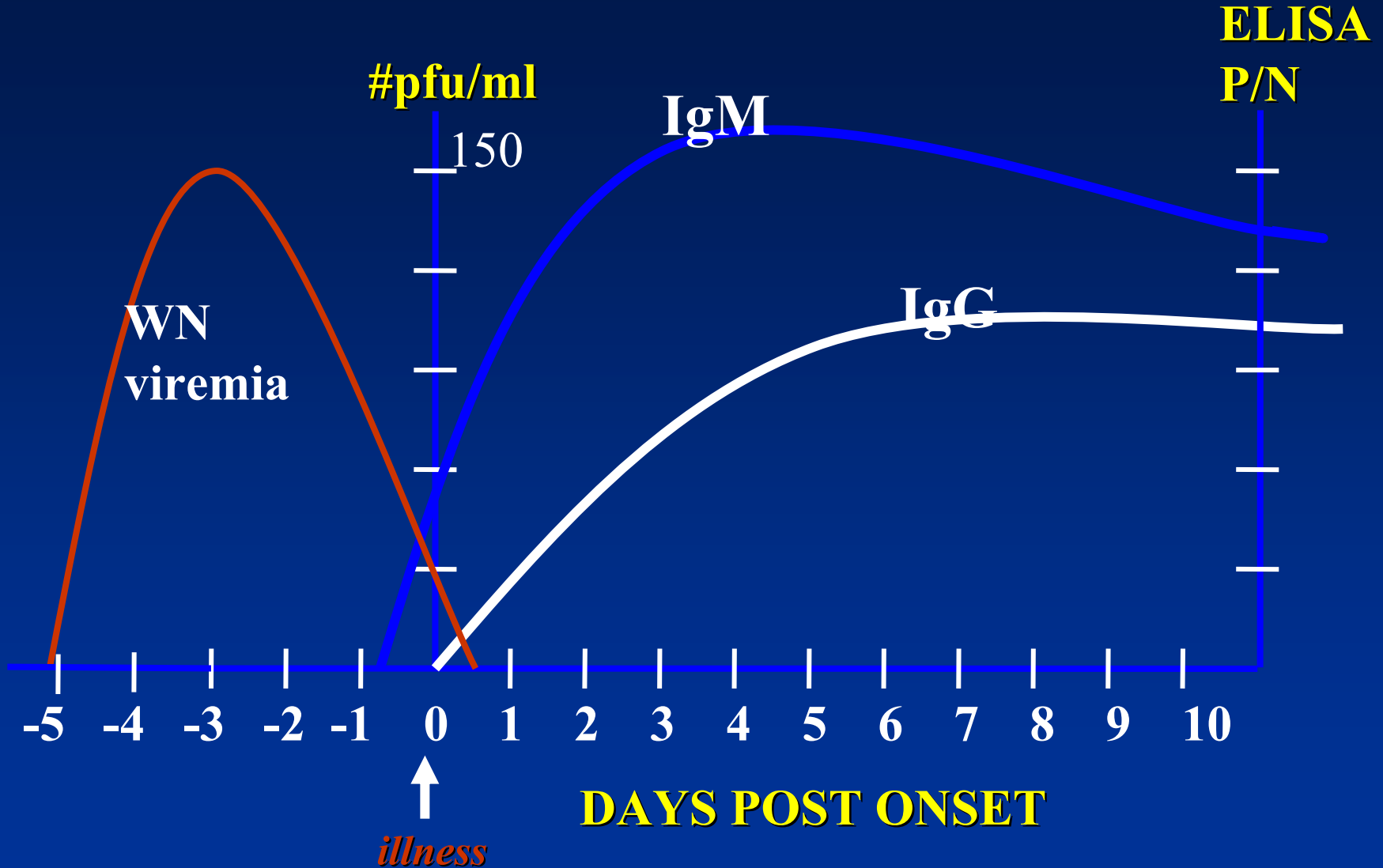


Dead end host

Laboratory Issues and West Nile Virus

- Arboviruses
- Laboratory Diagnosis
- Investigation of 2002 WNV outbreak in Michigan
- Plans for 2003-Lab perspective

Immune Response in WNV Infection



Ref: 4th National WNV Conf. 2003 www.cdc.gov

Laboratory Diagnosis of Human cases

- **Specimens**
 - **Cerebrospinal fluid (CSF)**
 - **CSF and Serum combination**
 - **Sera- Acute and Convalescent (obtained at least 8 d and 22 d post onset respectively)**
 - **Least preferred single serum sample**

Ref: Antibody Capture ELISA (IgM & IgG) Protocol. CDC Fort Collins, colorado

CDC Neutralization Test Protocol. CDC Fort Collins, colorado

Laboratory Diagnosis of Human cases contd

- **Laboratory Tests**

- **Capture enzyme-linked immunosorbent assay (MAC-ELISA-IgM).**

- **Capture enzyme-linked immunosorbent assay (MAC-ELISA-IgG) and**

- **Plaque Reduction Neutralization Test (PRNT)**

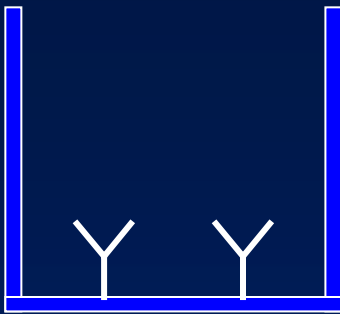
Ref: Antibody Capture ELISA (IgM & IgG) Protocol. CDC Fort Collins, colorado

CDC Neutralization Test Protocol. CDC Fort Collins, colorado

IgM Capture ELISA

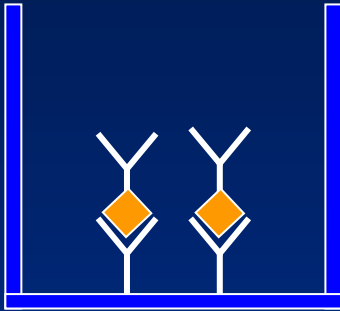
1. Coat With Goat anti-Human IgM

➤ 4° Overnight



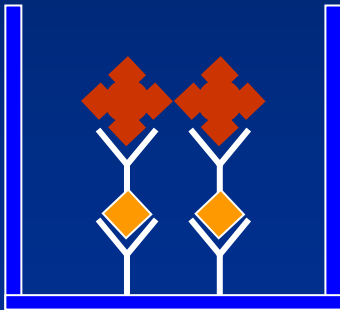
2. Add Patient Serum @ 1:400

➤ 37° 1 Hour



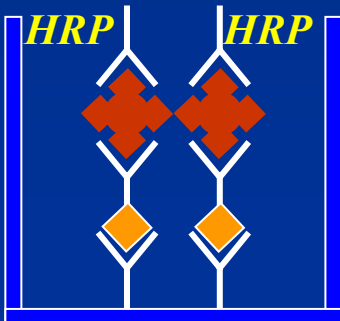
3. Add West Nile Recombinant Antigen

➤ 4° Overnight



4. Add HRP anti-Flavivirus McAb

➤ 37° 1 Hour



Ref: 4th National WNV Conf. 2003 www.cdc.gov

WN Serological Data

Typical Human WN Case

Sample	Days post-onset	IgM P/N		IgG P/N		PRNT	
		WN	SLE	WN	SLE	WN	SLE
<u>Typical WN Case</u>							
acute serum	8	12.75	4.00	1.37	2.04	1:80	1:20
conv. serum	31	11.35	4.21	6.38	5.76	1:1280	1:80

In primary flavivirus infections ;

➤ *Martin et al 2002: IgM P/N to WN is 3-5X greater than SLE.*

➤ **2002 data: Use 2X criteria WN to SLE ratio: only 1 exception in 417 WN confirmed cases.**

Ref: 4th National WNV Conf. 2003 www.cdc.gov

Serological Testing Criteria at Michigan

CSF	Paired sera (Ac. at least 8 days and conv at least 22 days post onset)	Single serum
<ul style="list-style-type: none"> •P/N ratio ≥ 10 positive •P/N 2-10 equivocal and requested for a serum sample. 	<ul style="list-style-type: none"> •P/N ratio ≥ 5 presumptive. •P/N 2-5 equivocal. •IgG Tests -A four-fold rise in titre to distinguish a recently acquired infection from a past infection. •PRNT was performed on all specimens showing a four fold increase in IgG titer. 	<ul style="list-style-type: none"> •P/N ratio- same as in paired sera. •A convalescent serum requested on equivocals . •PRNT To rule out the cross-reactions between WNV and other arbovirus infections (SLE, EEE and CGV).

•MAC-ELISA IgM performed in Singlet. Positive MAC-ELISA repeated in duplicate

West Nile Virus Nucleic Acid Amplification Tests (NAAT) Diagnostic Testing

- Highly persistent in the blood of WNV RNA positive window phase or convalescent patients
- Low levels WNV RNA found in clinical specimens with better sensitivity with better quality of diagnostic samples or virus concentration methods?
- WNV RNA(-) results are meaningless

Laboratory Issues and West Nile Virus

- Arboviruses
- Laboratory Diagnosis
- Investigation of 2002 WNV outbreak in Michigan
- Plans for 2003-Lab perspective

Weekly WNV Testing (Aug-Nov 02)



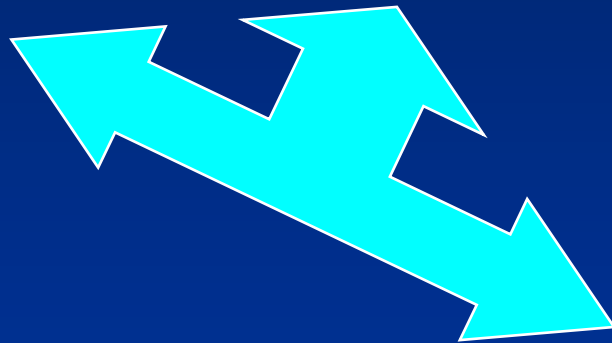
Total Specimens (Jan, 02-Dec, 02)-2910



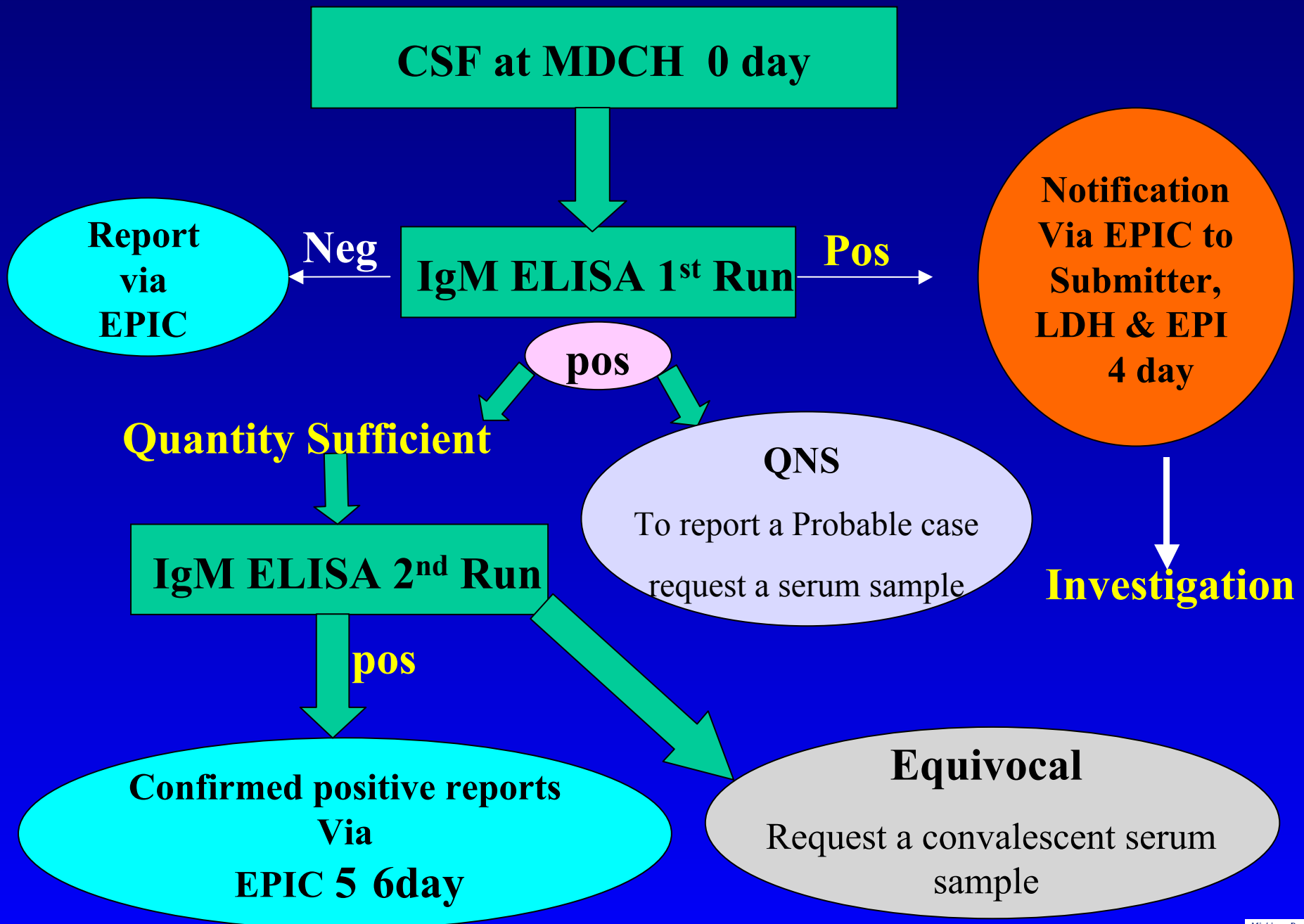
How we handled the 2002 outbreak in the laboratory ?

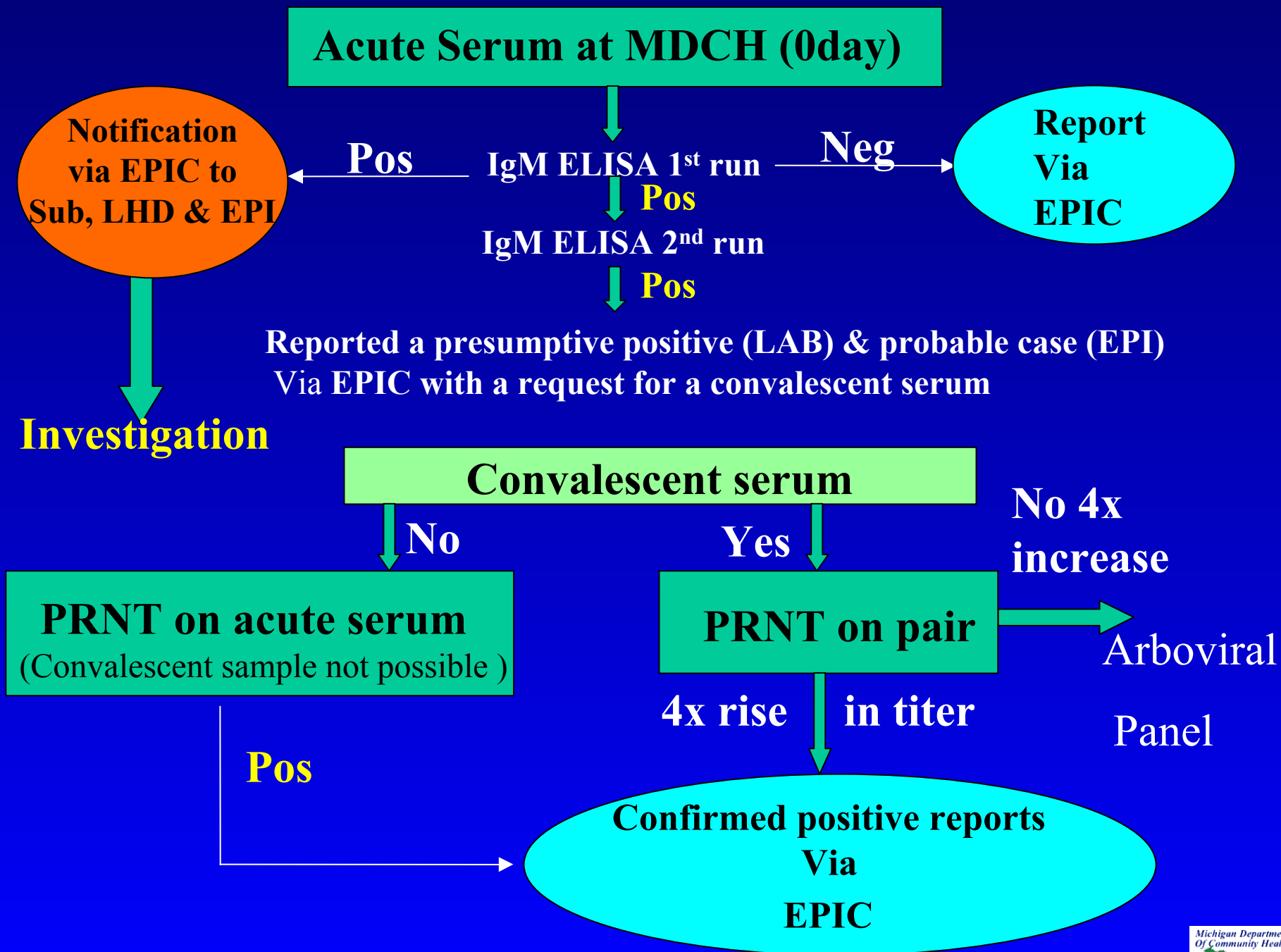


Triaged the samples

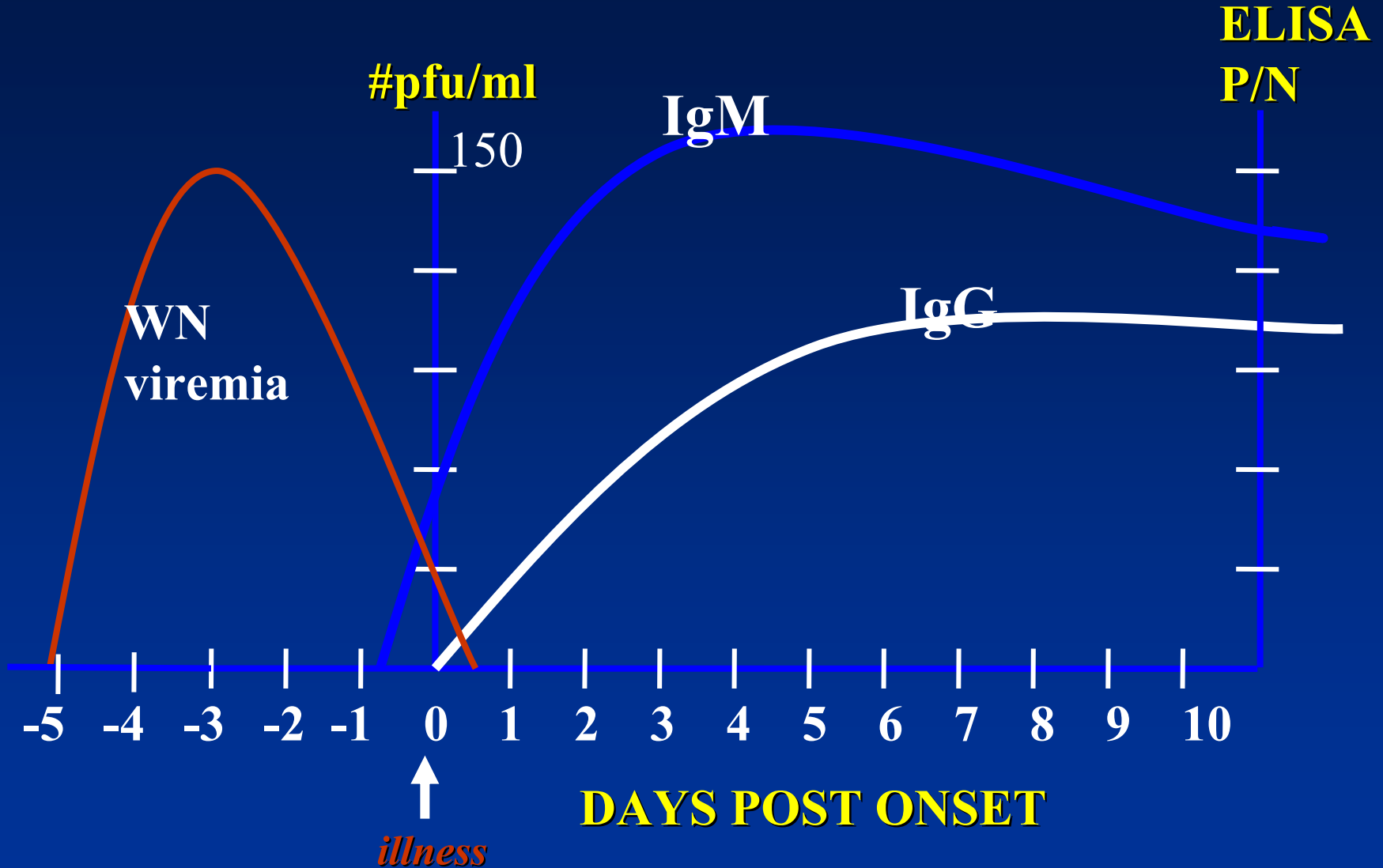


Virology section and intersection staff
worked collectively 6 days a week





Immune Response in WNV Infection



Ref: 4th National WNV Conf. 2003 www.cdc.gov

**Single serum with documented CNS symptoms
or paired sera without 4X increase in titer**

IgM ELISA (+) PRNT (-)

Test for SLE, EEE and CGV

Neg

**Single serum collected too early
(0-8 d) post onset**

Paired sera

Yes

No

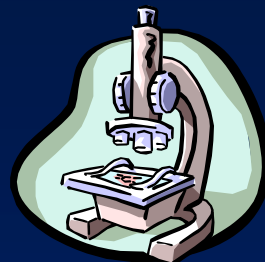
Yes

Probable WNV Case

No WNV Case

Laboratory Issues and West Nile Virus

- Arboviruses
- Laboratory Diagnosis
- Investigation of 2002 WNV outbreak in Michigan
- Plans for 2003-Lab perspective



Getting Ready for summer of 2003



WNV Surveillance in Michigan



MDCH - The Michigan Department of Community Health MDA- Michigan Department of Agriculture MDNR- Michigan Department of Natural Resources MSU- Michigan State University AHDLC- Animal Health Diagnostic Laboratory

Plans for 2003

Human Surveillance

- No triaging of samples
- Test whole panel
- Two LT positions in virology for WNV testing
- Cross training
- IgM capture ELISA testing on CSF and Paired serum
- Attempt culture on for WNV

Testing reagents for 2003

CDC Reagent Production

1995 - 1999	100 – 150 Reagent Requests/year
2002	560 reagent requests

- No change in personnel or policy
- Commercial Partners – patent license agreements for WN antigen production.

Manufacturers with WNV Antibody Assays in development

- Focus Technologies
- Ortho Clinical Diagnostics
- Abbott Laboratories
- Chiron (recombinant antigens)
- Pan -Bio

Future of new assays for WNV

Synergy between

- Industry
- Regulatory agencies
- Public and
- Private laboratories

Getting Ready for summer of 2003 contd

Issues with Persistence of IgM antibodies

Longevity of Human WN Virus-Reactive IgM in Serum

Days P.I.	N	Positive MAC-ELISA		Total (%)	Ave. P/N (Range)
		Positive (%)	Equivocal		
200	22	13 (60)	4	17 (77)	6.0 (3.0-10.8)
300- 400	21	9 (43)	2	11 (52)	4.0 (3.1-6.5)
500	12	5 (42)	2	6 (60)	5.0 (3.1-6.9)

Ref: 4th National WNV Conf. 2003 www.cdc.gov

2003 Testing

Acute Serum at MDCH

Hold and request for the

Convalescent serum

No

CNS symptoms +

Yes

PRNT (IgG) on pair

**No 4x
increase**

**Arboviral
Panel**

Approval of Virology Lab Manager

Report ← **Neg** ← **IgM ELISA 1st Run**

Pos
IgM ELISA 2nd Run

Pos

PRNT on acute serum

Pos

**4x rise
in titer**

**Confirmed positive reports
Via
EPIC**

Persistence of IgM antibodies

➤ In CSF-No studies

Published-47d

MDCH observations-Three cases with igM positive

➤ 110d

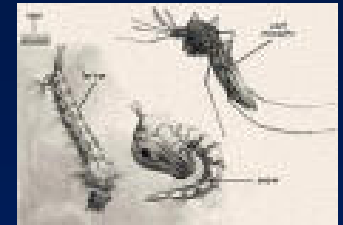
➤ 141d

➤ 199d

Bird Testing in 2003

- Strict IATA regulations
- Availability of a field assay-Vec Test
- Validation of IHC and PCR-2002
- IHC vs Vec Test at MSU lab in 2003
- Implementation of vec test ??

Over
wintering
adult *Culex*
mosquitoes



To Sum Up.....



**Human
Cases ?**

